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1	Year	Last	First	Dept.	Chair	Title	
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11253	1986	Oulton	Sarah	Zoology		The Role of Marek's Disease-Associated Tumor Surface Antigen (Matsa) as an Antigen Associated with JMV-1, in Immunization Against JMV.	
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THE ROLE OF MAREK'S DISEASE-ASSOCIATED TUMOR SURFACE ANTIGEN
(MATSA) AS AN ANTIGEN ASSOCIATED WITH JMV-1, IN IMMUNIZATION
AGAINST JMV

An Honors Thesis Presented

by

SARAH ANN OULTON

Submitted to the Zoology Departmental Honors Program of
the University of Massachusetts in partial fulfillment of the
requirements for the degree of

BACHELOR OF SCIENCE

May 1986

Department of Zoology

This senior honors thesis is dedicated to Donald and Carol
Oulton, my parents, for their love, support, encouragement, and
for their spirit of optimism.

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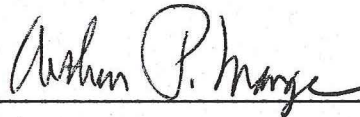
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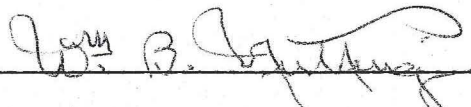
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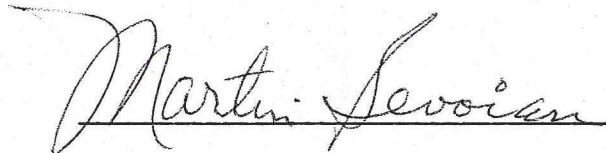
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ABSTRACT

THE ROLE OF MAREK'S DISEASE-ASSOCIATED TUMOR SURFACE ANTIGEN (MATSA) AS AN ANTIGEN ASSOCIATED WITH JMV-1, IN IMMUNIZATION AGAINST JMV

May 1986

SARAH ANN CULTON

B.S., University of Massachusetts

Directed by: Professor Martin S. Sevoian

A mouse monoclonal antibody against Marek's disease-associated tumor surface antigen (MATSA) was used to investigate the role of MATSA as an antigen associated with the JMV-1 lymphoblastoid cell line in immunization against the lethal transplant, JMV. Shortly after immunizing a day-old Cornish/White Rock cross of stock meat birds with 10^4 JMV-1 cells or with cell-free supernatant from 10^6 JMV-1 cells, each of which had been incubated with 50 uL/chick mouse monoclonal anti-MATSA antibody (M-MAb α MATSA) at concentrations of 10^{-2} , 10^{-3} , or 10^{-4} , specific deaths occurred in 3/5 birds. Three chicks from the group immunized with 10^4 JMV-1 cells incubated with 10^{-2} M-MAb α MATSA succumbed before they were to be challenged with JMV. They exhibited lesions typical of Marek's disease (MD) infection, i.e., erythematous livers, hepatomegaly, and splenomegaly. Inoculation of additional Cornish/White Rock cross chicks with varying numbers of JMV-1 cells incubated with varying concentrations of M-MAb α MATSA indicated a

predilection of MD lesion-exhibiting deaths to the groups of chicks inoculated with both 10^4 and 10^5 JMV-1 cells, incubated with either 10^{-1} or 1:20 M-MAb α MATSA. Genetically susceptible and resistant S and K line chicks were inoculated similarly, and were also inoculated with JMV-1 cells plus rabbit polyclonal anti-JMV-1 serum (R α JMV-1.) The chicks were challenged with 1000 chick lethal doses (CLD) of JMV 15 days after immunization. The results of these experiments suggest that the presence of intact MATSA on JMV-1 cells is required in order for the cells to afford protection against JMV, and also that the absence of MATSA renders the JMV-1 cells pathogenic.

CHAPTER I

INTRODUCTION

Leukosis is an abnormal proliferation of one or more of the leukopoietic tissues, and the term includes myelosis, certain forms of reticuloendotheliosis, and lymphadenosis. Avian leukosis is a complex of conditions (e.g., lymphoid leukosis, erythroid leukosis, myeloid leukosis) that occur chiefly in chickens and are characterized by an abnormal proliferation of myelopoietic or lymphoid tissues; some forms of the condition are apparently transmissible.

Within the avian leukosis complex is Marek's disease (MD) also known as Type II leukosis. It is a highly contagious, lymphoproliferative, neoplastic affliction caused by a cell-associated group B herpesvirus. The disease is a naturally-occurring, multifaceted disorder of fowl characterized not only by mononuclear cell infiltrations but also by lymphomas which may affect nerves, muscle, skin, and visceral organs. Its complex pathogenesis implicates MD as one of the most serious diseases of poultry and one of the most intriguing neoplastic diseases in any species.

The field of research on oncogenic herpesviruses has been led by studies on MD, the most notable contribution to which has been the development of vaccines. These are the first examples of a practical and effective immunological control of any cancer, in any species.

CHAPTER II

LITERATURE REVIEW

The History of Marek's Disease

The first account of MD was given in 1907 by the Hungarian Josef Marek, when he described inflammatory nerve lesions found in chickens as polyneuritis. Additional studies made by workers around the world during subsequent years established that in addition to paralysis and paresis, lymphomas may also manifest during the course of disease. Marek described MD as consisting of both neoplastic and inflammatory features (Calnek, 1980.)

Confusion existed as to the true nature of the disease, so for several decades it was grouped with other avian afflictions under the heading Avian Leukosis Complex. In addition, it was unclear over MD persisted as to whether the lesions were inflammatory or neoplastic, because both types occurred in affected nerves. Studies on transmission of MD provided further confusion, when experiments using blood or tumor cells from affected chickens gave equivocal results.

The most intensive research on the nature of the disease has been underway since the 1960s, led by the independent discoveries by Sevoian et al. and Biggs and Payne that MD could be regularly transmitted using cellular inocula of genetically susceptible chickens. These studies have led to the virtual control of the disease by vaccination, although complete eradication of it has not been achieved. In addition, they have provided a model for the study of oncogenic

herpesvirus infections, and have indicated that vast amounts of information on such infections remain to be learned.

Incidence, Distribution, and Economic Significance

MD is primarily restricted to chickens, although natural infections have been detected in other Galliformes, especially in the Gallus species. Experimental infections have been induced in ducks, turkeys, Japanese quail, and pheasants (Calnek, 1980) although often without disease. In short, MD may be regarded as the most common clinical neoplastic condition of any animal, and it occurs in poultry-producing countries throughout the world. An interesting aspect of the worldwide distribution of MD is that the disease is most readily recognized, and prevented through vaccination, in the most developed countries of the world. Table I is a partial summary of the presence and prevalence of MD in both developed and developing countries (Purchase, 1985.)

TABLE I A partial summary of the presence and prevalence of MD in
both the developed and developing countries of the world

a. Developed Countries

<u>Not Recorded</u>	<u>Rare</u>	<u>Low</u>	<u>Moderate</u>	<u>High</u>
Yugoslavia	Canada Belgium E. Germany	Denmark United States Hungary Italy Netherlands Poland Romania Spain Sweden United Kingdom U.S.S.R. W. Germany South Africa	Austria Australia Bulgaria Czechoslovakia France Greece Israel Japan Portugal	

b. Developing Countries

<u>Not Recorded</u>	<u>Rare</u>	<u>Low</u>	<u>Moderate</u>	<u>High</u>
Cuba Turkey	Thailand	Argentina Columbia Egypt Indonesia Phillipines Vietnam	Brazil Korea Mexico Peru Venezuela	India Iran Nigeria

One should note that the detecting and recording systems vary greatly, so it is unlikely that any country, including Cuba, Turkey, and Yugoslavia, is free of the disease (Purchase, 1985.)

Epizootiology of MD has demonstrated transmission to be strictly horizontal, thus vertical transmission through the egg from hen to progeny does not occur (Calnek, 1980.) Because of this MD is of lower prevalence under pastoral, extensive husbandry practices, although one must note that in this case the chicken is more likely to succumb to predation and is also less frequently observed. Under intensive husbandry there is considerable dust, waste, and feathers left in the houses between crops of chicks, and since infectious virions are shed only from the feather follicle epithelium (FFE), the result is early infection of young birds (Purchase, 1985.)

Prior to vaccine use the morbidity and mortality in egg-laying flocks ranged from 0-60%, with losses of up to 30% common. Incidence among meat-type chickens ("broilers" in the U.S.) was generally lower because they were usually slaughtered at 7 weeks of age. Today these levels of loss have been reduced in the developed countries but are still reached in the developing ones.

The first available vaccine against MD utilized the herpesvirus of turkeys (HVT), and was put to use in the 1970s. Various other vaccine types are also available, including a recently-approved bivalent vaccine consisting of HVT and an apathogenic strain of MDV. The total cost of MD is estimated to be \$160.2 million. In broilers this figure includes the cost of vaccination and loss from mortality

and condemnation (\$50 million.) In egg-laying chickens this represents a loss from mortality and a loss of egg production of \$110.3 million. The total worldwide cost of MD is very conservatively estimated to be \$943 million (Purchase, 1985.)

Clinical Signs of Marek's Disease

Infected birds experience one of the three clinical forms of the disease. Classic MD, the neurological disease described by Marek, occurs typically between 2 and 12 months of age. Although the specific clinical signs depend on the peripheral nerves involved, they include visceral lymphomas, paralysis or paresis of the legs and wings, torticollis, and weight loss. Acute MD is a more virulent form which affects chickens of 6 weeks or less, and its dominant feature is lymphoma formation of the visceral organs. Transient paralysis is a third form of MDV infection, occurring in birds between 5-18 weeks of age. In this acute encephalitis, apparently healthy birds develop paralysis or incoordination, with relatively low mortality (Powell, 1985.)

Pathogenesis

Figure I schematically represents the sequential events in the pathogenesis of MD. Infected birds remain carriers for life, shedding infectious, enveloped virions from the FFE. Virus is often associated with dead keratinized cells or attached to molted feathers, and it can remain infectious for months or years. The usual means of entry of MDV is by inhalation, although virus replication does not occur in the

respiratory tract. Within 3 days virus replication occurs in the bursa of Fabricius, the thymus, and the spleen. Macrophages are thought to play a role in the transport of virus from the respiratory tract to these lymphoid organs. In splenectomized birds infection of the bursa and thymus is delayed, suggesting spleen cells' importance in early replication and dissemination of virus (Powell, 1985.)

The infection of lymphoid tissues is acute and cytolytic, and during the peak infection (4-6 days post-exposure) large quantities of virus internal antigens (cytoplasmic and nuclear) are detected. Cell-associated (but not cell-free) virus may be isolated, and a number of spleen cells carry the Marek's disease-associated tumor surface antigen (MATSA.)

Early infection results in a necrotization of organs leading to acute inflammatory changes, termed acute reticulitis. A characteristic of MD is a proliferation of lymphoid cells in tissues and various organs, an accumulation that is particularly common to peripheral nerves (Sharma, 1980.) The end result is atrophy of the bursa and thymus, and consequently immunosuppression. The response of the spleen is one of reticular cell hyperplasia, resulting in splenomegaly.

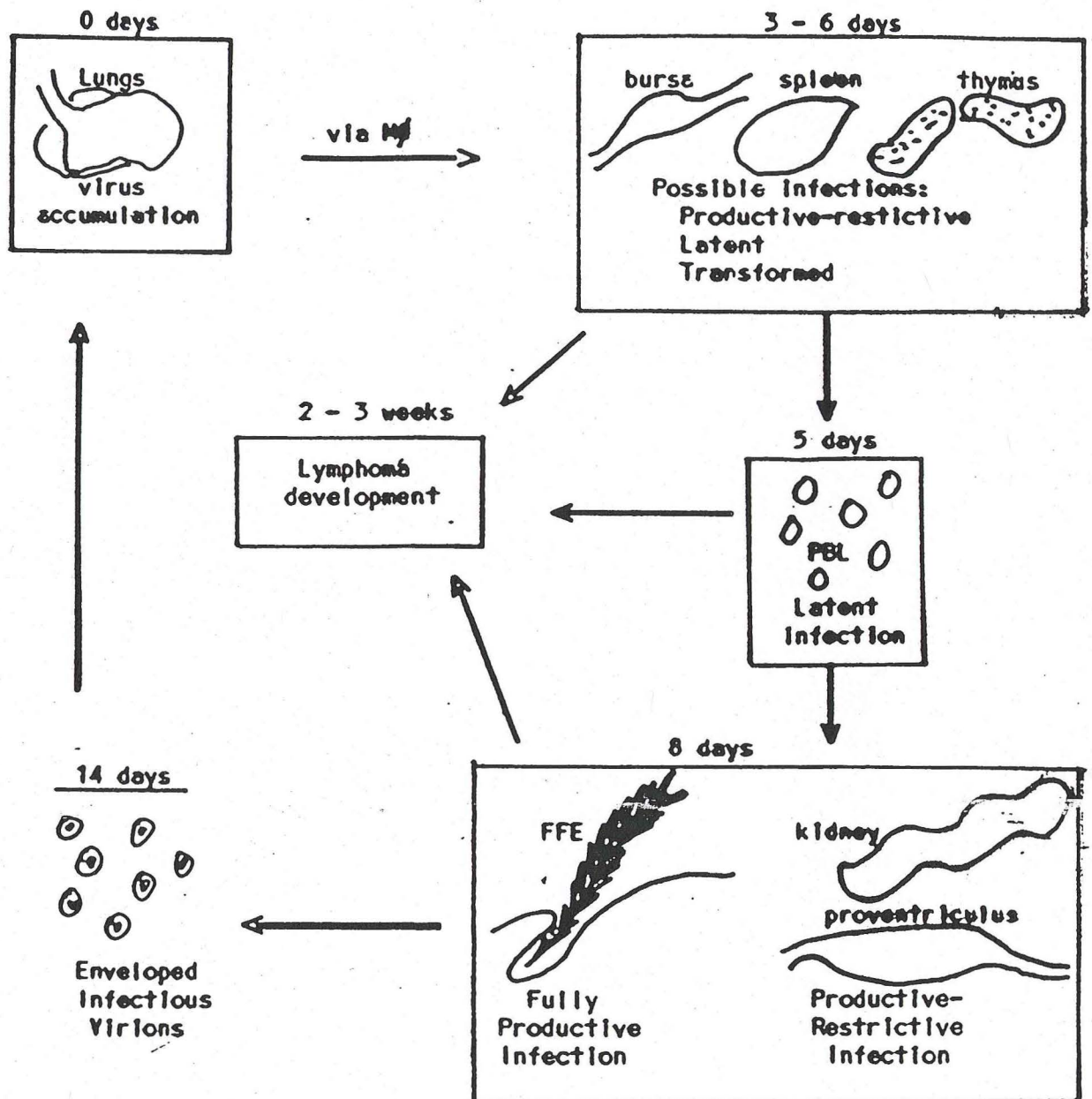
As shown in Figure I, cell-associated viremias associated with peripheral blood lymphocytes (PBL) are detectable from the fifth day post-exposure onward. During the second week of infection virus activity wanes in the lymphoid organs, and tissues of epithelial origin become infected. It is at this time that fully infectious, cell-free, enveloped virions are released from the FFE.

FIGURE I Schematic representation of the pathogenesis of Marek's disease (from Calnek, 1980)

MO = macrophage

PBL = peripheral blood lymphocyte

FFE = feather follicle epithelium



Characteristics of Marek's Disease Virus

A number of isolates of MDV have been described, a listing of which is presented in Table II (Schat, 1985.)

Isolates of serotype 1, the highly pathogenic strains, induce incidence of lymphomas with relatively brief incubation periods. The classic strains of MDV are the moderately pathogenic isolates of serotype 2, and they tend to induce a high incidence of neural lesions with relatively little lymphoma formation. This group is thought to be nononcogenic. The isolates of serotype 3 include the cell-associated lymphotropic HVT.

Oncogenic MDV isolates, nononcogenic MDV isolates, and HVT all share a number of common antigens, allowing for the use of HVT and nononcogenic MDV (of serotypes 3 and 2, respectively) as effective vaccines against the oncogenic serotype 1 isolates of MDV. Witter (1985) described two variants of MD-vaccine virus, which did not induce strongly protective immune responses to virulent MD challenge. The variants were thus determined to be unsatisfactory as commercial vaccines.

TABLE II Isolates of MDV and HVT grouped according to serotype

<u>Virus Type</u>	<u>Serotype</u>	<u>Oncogenicity</u>	<u>Representative Isolates</u>
-------------------	-----------------	---------------------	--------------------------------

MDV

1

+++

Md/5
Ala-8
RB-1B
Md/11

++

GA
HPRS-16
JM

+

HPRS-17
Conn-A

±

Cu-2
CVI 988

2

-

HPRS-24
SB-1
HN-1

3

-

FC 126
WTHV-1
HPRS-26

Studies on viral morphology demonstrate a nucleocapsid of 95-100 nm, having 162 hollow-centered capsomeres. There are small particles found in an infected cell nucleus 8 hours post-infection, which are positive for MDV antigen and cannot be labelled with ^3H -thymidine; thus they are protein and not DNA in nature (Schat, 1985.) Six of these particles fuse into a cylindrical mass in a capsid, around which spools of double-stranded viral DNA repeatedly coil. The first nucleocapsids may be found 10 hours post-infection; 8 hours later, the first enveloped virions may be seen.

Virus-Cell Interactions

a. Infection and Replication

The morphological and biochemical similarities among oncogenic isolates of MDV, nononcogenic isolates of MDV, and HVT are similar to those of other herpesviruses.

In permissive cells, enveloped virions may enter via the process of adsorption and penetration, a route common to herpesviruses. Intracellular naked virions are found approximately 10 hours post-infection, and enveloped virions appear 8 hours later. The envelopment process occurs at the nuclear membrane during a budding process through the inner nuclear membrane (Calnek, 1980.) Virus is released only from the FFE, in a passive release process which results from the normal breakup of keratinizing cells.

b. Types of Infection

In general , MD viral infections may be regarded as either productive or nonproductive, distinguished by the presence or absence of viral DNA synthesis, viral proteins, and virions. Productive infections may be either fully productive or productive-restrictive, depending on whether fully infectious virus is released by the cell. In both instances cell death results. Nonproductive infections may be either latent with no apparent genome expression, or may exhibit limited genome expression, resulting in a state of transformation.

c. The JMV-1 Lymphoblastoid Cell Line

Continuous lymphoid cell lines have been established from MDV-induced tumors and from transplantable lymphomas. There is a change in lymphoblastoid morphology after a variable (13-90 day) period in culture, and the target cell for transformation is the T-lymphocyte (Calnek, 1980; Powell, 1985.) Multiple copies of the MDV genome have been shown to be present in MD tumor cells, and there is a definite link between the presence of virus DNA and the transformed state (Calnek, 1980.)

Two methods proposed by Payne could account for the presence of neoplastic tumors (Powell, 1985.) One, the "intrinsic mechanism" implies that the virus genome directs transformation of the cell. Another, the "extrinsic mechanism" requires that lymphocyte proliferation be a direct result of an intense immune response to the viral antigens present on productively infected cells.

In 1964 Sevoian rapidly passaged the James Martin (JM) strain of MDV through susceptible chickens and thus developed JMV, the transplant-able lymphoblastic leukemia (Munch, et al., 1978.) The rapid in vitro passage resulted in a significantly reduced incubation period and increased mortality from MD, so JMV has since been used as an effective challenge strain in studies of vaccination against MDV. Using JMV, the JMV-1 lymphoblastoid cell line was developed in 1977 by Munch, et al. This in vitro cell line was developed from spleens of JMV-inoculated chicks, and although several genome copies of MDV were demonstrated in the cells, the line was found to be nonproductive of virus. In addition, no viral proteins were serologically detected in the cells, nor could virus be recovered from kidneys of JMV-1-inoculated chicks, or from co-cultivation of JMV-1 cells with duck embryo fibroblasts. This is crucial to the unusual nature of the cell line.

In 1980, Munch and Sevoian demonstrated that immunization of day-old chicks with attenuated JMV-1 cells resulted in a notable reduction in tumor development and in the amount of recoverable virus following challenge with JMV, when compared with non-immunized, JMV-challenged chicks.

In 1985 Keller and Sevoian demonstrated the usefulness of cell-free JMV-1 culture supernatant as a non-infectious and nononcogenic immunizing agent against MMDV infection and associated tumor development. They reported that the supernatant apparently stimulates a heightened humoral response to MDV, which in turn decreases the amount of virus in the chick. The result is a decrease in the amount of

consequent lymphoma development. The study implied the presence of protective antigens in the culture supernatant, and it presented the interesting potential for the development of a non-infectious and non-oncogenic immunizing agent against MD.

Virus-induced Antigens

Three regularly-recognized virus related antigens (known as A, B, and C antigens) are detectable in permissive cells infected in vitro, in lymphoblastoid cell lines, in FFE, and in tissues of the bursa, thymus, and spleen of chickens (Powell, 1981.) All three antigens are associated with infected cells, although only the C antigen is MDV-specific. The A and B antigens are common to both HVT and MDV, however. Via two-dimensional gel patterns of virus-specific polypeptides of several HVT and MD strains it has been possible to distinguish the three groups of antigens: a) high and low-virulent strains of MDV and their attenuated variants, b) avirulent and non-oncogenic MDV strains, c) HVT strains. Infected cells also contain virus-induced enzymes, such as a virus-specific DNA polymerase and an HVT-induced thymidine kinase (Powell, 1981; Lee, et al., 1985.)

Immunofluorescence on cultured cells has elucidated the distribution of viral antigens, and 4 antigen types have been described: a diffuse cytoplasmic antigen, a diffuse nuclear antigen, a granular cytoplasmic antigen, and a membrane antigen on infected cells. Payne, et al. demonstrated two types of membrane antigens: an early-appearing antigen detected prior to replication of the viral genome, and a late-appearing antigen that is dependent on a late function of the viral genome. The latter is closely related to virus envelope antigen, yet both are antigenically distinct (Powell, 1985.)

Marek's Disease-Associated Tumor Surface Antigen (MATSA)

Cells of MD-derived lymphoblastoid cell lines express an antigen

on the membrane that is also carried by a proportion of the cells present in MD lymphomas, yet is distinct from the viral antigens of productively or abortively infected cells (Powell, 1981.) Witter, et al. have termed it "Marek's disease tumor-associated surface antigen." MATSA is believed to be a marker for neoplastic transformation, and is induced not only by virulent MDV but also by attenuated MDV, non-oncogenic MDV, and HVT (Lee, et al., 1983; Powell, 1985; Schierman, 1985.)

Calnek (1980) notes the discovery by Ross, et al. that the major thymus-specific polypeptide found on normal cells is either not exposed or is absent from the surface of the transformed T-lymphoblasts. Thus, the question has been raised as to whether MATSA is an altered T-cell marker.

MATSA has been characterized as a 40,000 molecular weight polypeptide, and it appears to be neither a thymus tissue antigen nor a virus-encoded antigen.

Lee, et al. (1983) reported immunologic studies showing MATSA to be a tumor-associated antigen that is distinct from the MD virus-induced antigens that are present in productively infected cells. MATSA is unique in that it can be distinguished from the major histocompatibility antigens, and it is unrelated to chicken embryonic fetal antigens. Interestingly, antigenic differences between MATSA from different cell lines have been observed. Antiserum prepared against MATSA unique to one cell line does not react with MATSA unique to another; yet all antisera react, to varying degrees, with MD tumor

cells.

Lee, et al. (1983) discovered an antibody-secreting hybrid-oma to MATSA, and designated it RPH-6. The hybridoma was produced by somatic-cell hybridization between the mouse myeloma SP2/0-Ag/14 and spleen cells from MSB1-immunized mice.

The availability of so specific an antibody to MATSA provides a basis for additional immunization trials to be done using both lymphoblastoid cells and cell-free culture supernatant. The results of such studies, in which MATSA is effectively eliminated while leaving the remaining antigens intact, would indicate whether MATSA's presence on JMV-1 cells and in cell-free supernatant is required in order for the cells and supernatant to protect chickens against Marek's disease. By determining MATSA's role in protection, i.e., its importance or its irrelevance, one could hope to determine the mechanism by which JMV-1 cells and culture supernatant afford protection to naive chicks.

CHAPTER III

OBJECTIVES

The objectives of this thesis were:

1) to confirm the results obtained by Keller and Sevoian (1985):

a. that cell-free supernatant of JMV-1 lymphoblastoid cell cultures affords significant protection against challenge with lethal JMV

b. that JMV-1 cells also afford protection when used as an immunogen in day-old chicks

2) that rabbit polyclonal anti-JMV-1 antibody ($R\alpha$ JMV-1) negates the above protection when it is incubated with either cells or supernatant prior to immunization.

3) to investigate the role of MATSA as an antigen associated with JMV-1 lymphoblastoid cells in immunization against JMV, by:

a. evaluating the effects of inoculation of JMV-1 cells incubated with mouse monoclonal anti-MATSA antibody (M-Mab α MATSA) on day-old chicks

b. immunizing day-old chicks with JMV-1 cells plus M-Mab α MATSA or JMV-1 cell-free supernatant plus M-Mab α MATSA, and challenging with lethal JMV.

CHAPTER IV

MATERIALS AND METHODS

a. Experimental animals

The genetically susceptible and resistant S and K line chickens used in these studies were obtained from departmental flocks, and were free of known pathogenic avian viruses. A Cornish/White Rock cross of stock meat cockerels was kindly provided by Mr. Carl Weston of Hubbard Farms, Walpole, New Hampshire. The cockerels were transported to the University of Massachusetts shortly after hatching. All experimental birds were wing-banded for identification, and were maintained in Horsfall Bauer isolation units.

b. Cell cultures

The JMV-1 cell line used in the experiments has been maintained in continuous passage by diluting cultures every 48 hours, 1:10 in RPMI 1640 media (GIBCO, Grand Island, New York.)

The media was supplemented with 5% tryptose phosphate broth, 10% heat-inactivated chicken serum, 8% fetal bovine serum, 10^{-3} mM B-mercapto-ethanol, 1mM sodium pyruvate, and penicillin, dihydrostreptomycin, and mycostatin at 10,000 units, 10,000 ug, and 2,500 units, respectively. The cells were provided with a 5% CO₂-in-air atmosphere, and were incubated at 37°C.

JMV-1 cell-free culture supernatant was obtained from 48- hour cell cultures, prior to passaging, and Trypan blue exclusion was done to determine viable cell numbers. The suspension cell cultures were centrifuged at 4,000 x g for 5 minutes, and the resultant culture supernatant was filtered through a 0.4 u porosity nitrocellulose membrane.

The lethal tissue transplant JMV was maintained by in vivo passage through S and K line chicks as previously described (Munch, et al., 1978.) Liver and spleen homogenates were titrated in 15-day-old chicks, and the LD₅₀ was determined by the method of Reed and Muench (Lenette, 1964.) From these data the appropriate JMV concentration that would yield 1,000 chick lethal doses (CLD) was determined. JMV was aliquotted in 1.5 mL plastic Nunc vials and stored in liquid nitrogen for later use as challenge material.

c. Mouse monoclonal anti-MATSA antibody (M-Mab α MATSA)

RPH-6 antibody of the IgM class was kindly provided by Lucy F. Lee of the USDA Agricultural Research Service, Regional Poultry Research Laboratory, East Lansing, Michigan. The antibody was predetermined to have a cytotoxic titer between 10⁴ and 10⁶. It was from this stock solution of M-Mab α MATSA that subsequent dilutions were made.

d. Rabbit polyclonal anti-JMV-1 antibody (R α JMV-1)

Rabbit-derived polyclonal antibody against the JMV-1 cell line was obtained from storage at -20°C, after having been prepared and stored as previously described (Keller and Sevoian, 1985.)

e. The effect of M-MAb α MATSA on JMV-1 cells

Day-old Cornish/White Rock cross cockerels and day-old S and K line chicks were separated into treatment groups and banded for identification. JMV-1 cell cultures were checked for numbers of viable cells and were centrifuged at $4,000 \times g$ for 5 minutes. The cells were resuspended in phosphate buffered saline pH 7.2, and recounted. Cells were then aliquotted into test tubes, containing 1.0×10^3 , 1.0×10^4 , or 1.0×10^5 cells per chick/group. Dilutions of M-MAb α MATSA were made at 10^{-1} , 1:20, and 10^{-2} , and amounts of 50 μ L per chick/group were added to the cell-containing test tubes. Incubation at 37°C for 30 minutes was conducted prior to inoculating the chicks. The experiment was repeated using 1.0×10^4 , 1.0×10^5 , or 1.0×10^6 cells per chick/group incubated with 50 μ L chick/group of either M-MAb α MATSA at a 1:20 dilution, or R α JMV-1 at a 10^{-2} dilution. In both studies, appropriate control groups were maintained.

f. Protection against lethal JMV

Immunization trials were conducted in day-old Cornish/White Rock cross cockerels and in day-old S and K line chicks, using varied numbers of live JMV-1 cells (10^3 - 10^6 per chick/group), cell-free supernatant from 10^6 JMV-1 cells, and either M-MAb α MATSA or R α JMV-1 incubated with the cells and supernatant. Appropriate control groups were simultaneously maintained. Fifteen days after immunization, chicks were challenged with 1,000 CLD of JMV and were observed for 15 days for JMV-specific mortality, which was determined by the presence of hepatomega-

ly, splenomegaly, and erythematous livers. All birds were necropsied.

CHAPTER V

RESULTS

Protection against lethal JMV by vaccination

a. Immunization experiment #1

150 Cornish/White Rock cross cockerels were wing-banded and separated into groups of 10. Each group was immunized with JMV-1 cells or cell-free culture supernatant, both of which had been incubated with various concentrations of M-Mab α MATSA. The results of this trial are presented in Table III. 30% of the chicks immunized with 10^4 JMV-1 cells plus 10^{-2} M-Mab α MATSA succumbed prior to challenge, and all exhibited lesions characteristic of MDV infection. This was the highest percentage of pre-challenge mortality of any group, and only these chicks displayed MD lesions at necropsy. The remaining chicks were challenged with JMV at day 15 post-immunization, and were monitored for specific deaths during the next 15 days. The JMV challenge in this trial was unsuccessful in that none of the chicks, including those of the "no treatment" group succumbed within the 15 day period.

TABLE III Immunization experiment #1: percentages of chicks exhibiting MD lesions post-immunization

	No Tx ^c	10 ⁴ JMV-1 cells	Supernatant of 10 ⁶ cells
No Tx	0.0	0.0	0.0
a 10 ⁻⁴	0.0	0.0	0.0
10 ⁻³	0.0	10.0	0.0
10 ⁻²	0.0	30.0	10.0
b 10 ⁻² NMS	0.0	0.0	0.0
10 ⁻² R JMV-1	0.0	0.0	0.0

a M-MAb α MATSA

b Normal mouse serum

c Tx =treatment

b. Immunization experiment #2

JMV stored in liquid nitrogen was removed and titered in 15-day-old chicks. The LD₅₀ was determined to be 10⁶. Liver and spleen homogenates were then made as chicks succumbed, and stored for later use as challenge material. S and K line chicks were wing-banded, grouped, and immunized according to the predetermined groups. As had occurred in immunization experiment #1, pre-challenge deaths occurred, but all chicks in this experiment were negative for MD lesions at necropsy. Challenge with 1,000 CLD of JMV was done at day 15 post-immunization, and the results of the trial are presented in Table IV.

TABLE IV Immunization experiment #2: percentages of immunized chicks
exhibiting MD lesions post-JMV challenge.

		NUMBER OF JMV-1 CELLS PER CHICK/GROUP					
		No Tx	10 ³	10 ⁴	10 ⁵	10 ⁶	Supernatant of 10 ₆ cells
No Tx		80.0	40.0	0.0	0.0	—	0.0
a	1:20	80.0	—	88.0	44.0	13.0	—
b	10 ⁻²	80.0	—	40.0	20.0	0.0	—
c	1:40	100.0	80.0	60.0	33.0	—	33.0
	1:80	50.0	80.0	0.0	20.0	—	33.0
	1:160	80.0	25.0	20.0	0.0	—	40.0
d	1:40	100.0	20.0	60.0	0.0	—	0.0

a M-MAb α MATSA

b Normal mouse serum

c R JMV-1

d Normal rabbit serum

e Tx = treatment

c. The effects of M-MAb α MATSA on JMV-1 cells

After observing a 30% mortality of chicks following immunization with M-MAb α MATSA-treated JMV-1 cells, additional chicks were inoculated with JMV-1 cells incubated with varied concentrations of M-MAb α MATSA. This was done in order to establish a pattern in the pre-challenge deaths, possibly resulting from a specific cell:antibody ratio. 80 Cornish/White Rock cross cockerels were used in this study, and the data from the experiment are presented in Table V.

In addition to this study, chicks involved in immunization experiment #2 were monitored for pre-challenge deaths. The results of this experiment contradict those observed in immunization experiment #2, where pre-challenge deaths which occurred were not coupled with MD lesions.

TABLE V Percentages of chicks exhibiting MD lesions following inoculation with JMV-1 cells incubated with M-MAb α MATSA

	No Tx	10 ³ a	10 ⁴	10 ⁵
No Tx	0.0	20.0	0.0	0.0
b 10 ⁻¹	0.0	0.0	80.0	60.0
1:20	0.0	0.0	80.0	60.0
10 ⁻²	0.0	0.0	0.0	20.0

a JMV-1 cells

b M-MAb MATSA

CHAPTER VI

DISCUSSION

The immune response of chickens infected with Marek's disease virus can be directed against both virus and tumor cell antigens, and an effective response against either one has been found to prevent the development of fatal lymphoblastic leukemia (Murthy and Calnek, 1979; Powell, 1985.) Non-productive in vitro cell lines developed from the lymphoblastic transplant JMV provide an intriguing opportunity to study not only the chicken immune response but also the effects of MDV-transformed lymphocytes on that response.

Since the virus-free nature of JMV has been established (Calnek, 1980; Powell, 1981), a study of the immune response of chickens to JMV-transformed lymphocytes is clearly a study free from the influences of viral antigens or replicating virus.

The results of this study tend to support the findings of Keller and Sevoian (1985) that culture supernatant from the non-productive JMV-1 lymphoblastoid cell line contains antigens protective to chicks against challenge with the lethal JMV transplant. The data also support the results of previous assays with JMV. Shieh and Sevoian (1975) found day-old chicks vaccinated with cell-free JMV were 100% protected against challenge with 1,000 CLD of JMV. Shieh and Sevoian (1974) also found that day-old chicks vaccinated with cell-free JMV developed serum neutralizing antibodies against JM virus. They demonstrated a significant reduction in virus recovery and tumor development

from the progeny of JMV-vaccinated dams, as did Hong and Sevoian (1974.) Munch and Seovian (1980) demonstrated the ability of JMV-1 to protect chicks against challenge with lethal JMV, and found that such immunization significantly altered the pathogenesis of MDV infection.

It is not known whether the observed protection against JMV is elicited against viral antigens, tumor antigens such as MATSA, or both. The ability of normal rabbit serum to block the protective response negates the possibility of a non-specific blockage of the protection afforded by JMV-1 cells. Immunization with JMV-1 cells and with cell-free culture supernatant apparently stimulates a heightened humoral response to MDV (Keller and Sevoian, 1985.)

The availability of the mouse monoclonal anti-MATSA antibody provides the opportunity to study the immunogenic properties of the JMV-1 cell line in greater detail, because it is now possible to serologically block only MATSA, while leaving the remainder of the JMV-1 cell intact. The data obtained in these studies implicate MATSA in the protection afforded to chicks by JMV-1 cells upon challenge with the lethal JMV transplant.

The occurrence of pre-challenge, MD lesion-exhibiting deaths in chicks inoculated with JMV-1 cells incubated with M-Mab~~α~~MATSA (immunization experiment #1) were, at first exciting, because they implied that MATSA's presence on the JMV-1 cells was crucial to the non-productive nature of the cells. However, because immunization experiment #2 did not yield similar pre-challenge results, the exact role of MATSA, indeed the exact cause of the results observed in immunization experi-

ment #1, remains ambiguous.

The results of immunization experiment #2 are significant because they show that MATSA is apparently involved in the protective nature of the JMV-1 lymphoblastoid cell line. In this experiment the effects of blockage of MATSA with M-MAb were greater than with the polyclonal R JMV-1, and it will be interesting to repeat the experiment in the future to confirm this finding.

Although the findings of these experiments are indicative of the essentiality of MATSA on the JMV-1 lymphoblastoid cell line in order for the cells to retain their protective nature, much work remains to be done. Repetition of these experiments will be helpful in confirming or denying MATSA's essentiality, and will be helpful in the exploration of the immune response of chicks to MDV protective antigens.

CHAPTER VII

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